THE EFFECTS OF SALTS AND SUGAR SOLUTIONS ON THE DEVELOPMENT OF THE FROG'S EGG.

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The purpose of these experiments was to determine more definitely what action takes place when eggs of the frog are treated with solutions containing both salts and sugar, as compared with control solutions containing only salt or sugar. We have also tried to compare the action of such sugars as cane sugar, that might possibly invert, with the action of simpler sugars, like glucose or lævulose. We have kept in mind the possibility that while the chief action of sugar is through its osmotic pressure, yet sugar may also act chemically on the living substance; or by forming new compounds with the inorganic salts may affect the results in this way.

Action of Lithium Chlorid and Sodium Chlorid Acting Alone.

The concentration of LiCl that will prevent the development from proceeding beyond the segmentation stages was previously determined for the egg of *Rana sylvatica* to be about 0.65 per cent. Since the salts obtained at different times may vary in the amount of absorbed water, it was necessary (for percentage solutions were used) to make a new determination as control for exact comparisons with the effects of the new salt when united in solution with sugars. The eggs were put into the solutions in the 2-cell stages in all cases. It was found that in LiCl 0.5 per cent. (the strength used in combination with sugar) the blastopore appeared and sometimes closed almost normally; usually it formed a ring around or below the equator of the egg. Lithium chlorid of this strength is, therefore, near the limit of inhibitory effects but below that limit. Some solutions of this strength were used with sugar solutions.

The upper limit of NaCl was not accurately determined in previous work on the frog [1] and the results are not in harmony with

[1] Morgan, T. H., "Experiments with Frog's Egg," Biol. Bull., XI., 2, 1906.

the present ones. In NaCl 0.5 per cent. development was nearly normal; in 1.0 per cent. only the 16- and 32-cell stages were reached; in 1.5 per cent. only the 16-cell stage, and in 2.0 per cent. only the 8-cell stage, and in the 2.5 per cent. only the 4- or 8-cell stage was reached, while in the 3.0 per cent. the eggs died without developing further. Similar results were obtained in another series of the same kind. In a third series of smaller range it was found that in a 0.5 per cent. solution the later cleavage stages were reached; in a 0.7 per cent. solution also only the late cleavage stages appeared; in a 0.9 per cent. solution the cleavage had not gone so far, while in a 1.0 per cent. solution only the 32- or 64-cell stages had been formed.

The upper limit for NaCl lies, therefore, somewhere between 0.5 and 1.0 per cent. and not above 2.0 per cent. as previously stated. This difference in the results may possibly have been due to some impurities in the NaCl, not present in the salt used the previous year. The osmotic pressure for 0.5 per cent. is 3.55 atmospheres and for 1.0 it is 6.96. The latter is above that of LiCl 0.65 per cent., which is 6.16.

Action of Solutions Containing both Salts and Sugars.

Previous work on the frog's egg had indicated that when to a salt solution, too weak in itself to prevent development, a certain amount of sugar is added the development may be prevented; and a comparison of the osmotic pressures showed that in such a solution the pressure is higher than that when the salt alone produces the same results, but lower than that necessary for the sugar alone to produce the effect. Similar results have been obtained for the salt-water fish, *Fundulus*, where the outcome is even more striking owing to the fact that the eggs of this fish will not develop in a fresh water solution of salt and sugar that has an osmotic pressure lower than that of sea water in which they normally develop.[1] We have gone over these results with the frog in order to make sure that the effects were not due to the inversion of the cane sugar previously employed (that would increase its osmotic pressure) or that the effects were not due to an adulteration of the cane sugar with other sugars.

[1] Stockard, C. R., "The Influence of External Factors, Chemical and Physical, on the Development of Fundulus heteroclitus," *Jour. Exp. Zoöl.*, IV., 2, 1907.

In a ten per cent. solution of cane sugar the blastopore may develop, but in a thirteen per cent. solution only the later cleavage is reached. In a 5.5 per cent. solution of glucose the blastopore may develop in an abnormal way, while in a 6.0 per cent. solution only the late cleavage stage is reached. The results show that the same effect is produced by the same osmotic pressure of the two sugars.

A double solution of LiCl 0.5 per cent. plus glucose 0.5, 0.7, and 1.0 per cent. gave the following results:

LiCl 0.5 per cent. plus glucose 0.5 per cent., late segmentation.
LiCl 0.5 per cent. plus glucose 0.7 per cent., late segmentation.
LiCl 0.5 per cent. plus glucose 1.0 per cent., segmentation more abnormal.

The action of LiCl 0.4 per cent. plus glucose 1.0, 2.0 and 2.5 per cent. was as follows:

LiCl 0.4 per cent. plus glucose 1.0 per cent., very late segmentation: abnormal blastopore.
LiCl 0.4 per cent. plus glucose 2.0 per cent., late segmentation; not so far.
LiCl 0.4 per cent. plus glucose 2.5 per cent., late segmentation.

The results show that by adding amounts of glucose to a solution of LiCl, the development is stopped at a pressure higher than that for LiCl alone, but less than that for glucose alone.

Another similar experiment gave the same results. In a third experiment with LiCl 0.4 per cent. plus glucose 0.5, 1.0, 1.5 and 2.0 per cent. the results were nearly the same as is shown below.

LiCl 0.4 per cent. plus glucose 0.5 per cent., gastrulation normal, but delayed.
LiCl 0.4 per cent. plus glucose 1.0 per cent., barely gastrulating: abnormal.
LiCl 0.4 per cent. plus glucose 1.5 per cent., barely gastrulating: abnormal.
LiCl 0.4 per cent. plus glucose 2.0 per cent., late segmentation only.

The upper limit for this combination is about LiCl 0.4 per cent. plus glucose 2.0 per cent. with an osmotic pressure of 6.63, which is above LiCl .65 (= 6.16), but lower than glucose 6.0 (= 8.376).

The results with NaCl plus glucose were as follows: In a solution of 0.5 NaCl plus glucose 1.0, 1.5, 2.0 and 3.0 per cent. the late segmentation stages developed, but the yolk was injured. In another experiment the results were more decisive.

NaCl 0.5 per cent. plus glucose 1.0 per cent., circular blastopore above equator.
NaCl 0.5 per cent. plus glucose 1.5 per cent., late segmentation.
NaCl 0.5 per cent. plus glucose 2.0 per cent., dead in segmentation stages.
NaCl 0.5 per cent. plus glucose 3.0 per cent., not so late segmentation.

The upper limit for this combination lies therefore about NaCl 0.5 per cent. plus 3.0 per cent. glucose.

The Action of Sugar Solutions.

The experiments with sugar solutions were conducted to find if possible an explanation of the peculiar results which Stockard had obtained during the past summer by treating *Fundulus* eggs with solutions of sugars and sugar and salt mixtures. When *Fundulus* eggs were subjected to a solution containing LiCl or NH_4Cl and cane sugar the action of the salt was greatly augmented by the presence of the sugar even though the osmotic pressure of the mixture was lower than that of sea water. This indicated that the more marked action was not due to any increase in osmotic pressure that may have resulted from the addition of the sugar, but to some further or new chemical action.

Equal amounts of sugar were found to exert a more injurious effect on *Fundulus* eggs when in fresh water than when in sea water, although obviously the osmotic pressure of the latter was much the greater. This seemed possibly to indicate that the cane sugar in the fresh water solutions had become inverted, thus producing these pecular results. From a consideration of the experiments below it would seem more probable, however, that sugar exerted some chemical action on the compounds of the egg when in fresh water solutions rather than that inversion had taken place resulting only in an increase of pressure.

Frog eggs when in the four-cell stage were subjected to the following solutions of cane sugar: 6, 8, 9, 10, 11, 12, 12.5, 13, 15, 17 and 20 per cent. Although this is a series of fairly wide range it was found that the eggs were only slightly affected in the 6 per cent. solution, while they reached a late segmentation stage even in the 15 per cent. solution; the limit of effectiveness or fatal dose of sugar is thus seen not sharply indicated as in the case of many salts where a small fraction of a per cent. difference in the concentration of the solution gives at the critical points a marked difference in the effects on the eggs. The specific gravity of the sugar solutions was so high in most cases that the eggs would float in an indifferent position, the greater weight of the yolk pole not serving as it normally does to orient the egg in a definite

manner. The sugar solution was freshly prepared before starting the experiments, and Heines' solution was used to test the sugar to further assure ourselves of its purity and uninverted condition.

The 6 per cent. cane sugar solution delayed the rate of development after about twenty hours, so that when forty-eight hours old the eggs were far behind the control; and although neural folds and other indications of the embryo were present the embryonic outline was usually shortened as an effect of the delayed blastopore closure. The 8 per cent. solution gave much more marked effects. After twenty-four hours abnormal gastrulæ were formed, though none became elongated or showed any indication of embryo formation. Such a condition is similiar to that described below for eggs in 4 and 5 per cent. solutions of glucose and lævulose; the pressures of the 5 per cent. solutions are, however, slightly more than that of the 8 per cent. cane sugar. After fifty hours all of the eggs in the 8 per cent. solution were dead. Cane sugar of 9 per cent. had much the same effect.

Solutions of 10, 11, 12 and 12.5 per cent. cane sugar gave rather uniform results. Development was delayed within ten hours or less and usually stopped before gastrulation had commenced. In the 10 per cent. solutions, however, some eggs formed very abnormal gastrulæ of a rather uniform type, the upper dark or micromere portion of the egg had sunken in the lower coarser cells suggesting somewhat in gross appearance an acorn held in its saucer-like burr. It is of interest to note that such a type of gastrula was also found in the 5 per cent. glucose and lævulose solutions which exert approximately the same pressure as the 10 per cent. cane sugar.

The 13 and 15 per cent. solutions act much the same, the weaker giving less marked effects than the stronger one. After ten hours the eggs were much delayed, the white area had not been encroached upon by the darker cells and was divided into only six or eight large blastomeres. The eggs were all much plasmolized and development after twenty-two hours in the solution had progressed only about as far as control eggs of nine or ten hours old. Late segmentation was reached, and the eggs died in this condition after forty-five hours.

The 17 per cent. sugar exerts a pressure of about 11.86 atmospheres above that of the normal medium in which these eggs live. The eggs were effected very readily under such conditions. After only six hours in the solution a few showed no cellular structure at all, while the others were far behind the control in their rate of development. All died in late segmentation stages.

The 20 per cent. cane sugar stopped the development of most eggs within six hours, the blastomeres seemed to have fused together. A very few eggs divided to about the sixth or seventh cleavage and then underwent cytolysis.

Lactose or milk sugar was tried but this substance dissolved so slowly that it was difficult to interpret the results, and since the maximum pressure of the solution was reached only after the eggs had developed much beyond the 4-cell stage, the effects are not readily compared with those resulting from the use of the other sugars.

Simple sugars, glucose and lævulose, which are the inversion products of the cane sugar molecule were tried in order to determine if possible whether solutions of these which were isotonic with a given cane sugar solution would give similar results. Approximate comparisons of these solutions may be made as follows: A 5 per cent. solution of glucose or lævulose exerts a pressure nearly the same as a 10 per cent. solution of cane sugar. It is not exactly the same since a molecule of cane sugar, $C_{12}H_{22}O_{11}$, is a little less than twice a molecule of glucose, $C_6H_{12}O_6$, and in addition to this it must also be borne in mind that equi-molecular solutions of glucose and cane sugar do not exert exactly the same osmotic pressures, although for general purposes the two are considered about equal.

Eggs when in the four-cell stage were placed in the following strengths of glucose, 2, 3, 3.5, 4, 5, 5.5, 5.8, 6, 6.5, 10 and 15 per cent. The 2 per cent. solution had a very weak action causing the development to proceed slower than usual. After forty nine hours in the solution many of the eggs were slightly abnormal.

The 3, 3.5 and 4 per cent. solutions retard development considerably within twenty hours, and those in the 4 per cent. solution show abnormal gastrulation with prominent yolk-hernias.

The eggs in the latter solution are also plasmolized. In all three solutions the eggs died after about fifty hours as abnormal gastrulæ.

Eggs in 5 per cent. glucose in one of the experiments, formed abnormal gastrulæ, which closely resembled those described above in the 10 per cent. cane sugar, but in other experiments only a few eggs attempted gastrulation, and the majority died while in late segmentation stages. In the 5.5, 5.8 and 6 per cent. solutions late segmentation was reached, but all eggs were badly plasmolized with the animal pole flattened. Eggs in a 6.5 per cent. solution died in much the same condition, though plasmolysis occurred earlier in this solution.

Eggs underwent only a few divisions after being subjected to the 10 per cent. glucose solution, the effect was much the same as that of the 20 per cent. cane sugar.

In the 15 per cent. glucose the eggs were readily killed, the blastomeres being ruptured after only one or two divisions, or within about one hour after being subjected to the solution. This serves to convey some idea of how quickly these high osmotic pressures produce an effect.

Lævulose solutions of 2, 3, 5, 5.5, 6, 6.5, 8 and 10 per cent, were used. The general effects of such solutions were almost identical with those described for the same percentage solutions of glucose, showing that the actions were in the main part due alone to their osmotic pressures, and not to any difference in chemical action which the sugars might have exerted. One would not expect the chemical action of these sugars to be marked even if it was at all perceptible.

A consideration of the responses of frog's eggs to sugars would seem to indicate that the more violent action on *Fundulus* eggs of fresh-water solutions of cane sugar when compared with sea-water solutions is not due to the sugar in the fresh water having become inverted. It will be recalled that *Fundulus* eggs are more susceptible to the same percentage solution of a salt in fresh water than in sea water. These facts together with the case before mentioned of the augmented effect produced when sugar is added to a weak solution of a salt in fresh water, even though the pressure of the solution is below that of sea water, go to

show that the results with these eggs are not due so largely to osmotic pressure, but more to some chemical action which appears to take place between the constituents of the egg substance and sugars or salts when contained in fresh-water solutions. It may be that something present in the usual medium in which they develop, the sea water, prevents to some extent such an action, thus both salts and sugar act less violently when applied in sea-water solutions. The physiological condition of the egg may be weakened in fresh water as is indicated by its slightly retarded development in this medium, and under such circumstances they may be more susceptible to external injurious influences.

It is possible that new substances may be formed when sugar is added to salt solutions since some salts, *e. g.*, NaCl may form sodium-sugar compounds. Such compounds might be more or even less toxic than the original chemicals from which they resulted. These suggestions, which are at best speculative, serve to show how far we are from an understanding of the manner in which eggs respond to chemical stimuli, and indicate the importance of obtaining more complete data on the subject.

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